

Prognosis and rescue therapy for sepsis-related severe thrombocytopenia in critically ill patients

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ABSTRACT

Sepsis is the most common critical disease with high mortality in intensive care unit. Platelet count (PC) frequently altered in sepsis patients and implicated in the pathogenesis of multi-organ failure. It is also worth mentioning that thrombocytopenia was closely associated with poor outcomes in sepsis patients. However, whether drug intervention aimed at correcting thrombocytopenia would improve the prognosis of sepsis patients and which kind of sepsis patients could benefit from this therapy is still unclear. This study aims to explore the effect of severe thrombocytopenia on the prognosis of sepsis and the impact of a platelet-elevating drug (recombinant human thrombopoietin, rhTPO) for these sepsis patients. In this study, we included 249 sepsis patients diagnosed by sepsis 3.0, and these patients were classified into the three groups based on PC: normal ($PC \geq 100 \times 10^9/L$), mild-moderate thrombocytopenia ($50 \times 10^9/L \leq PC < 100 \times 10^9/L$), and severe thrombocytopenia ($PC < 50 \times 10^9/L$). We found that patients with severe thrombocytopenia had more blood transfusion, shorter days free from organ support, and worse outcomes as compared with the normal group. However, there was no significant difference between normal and mild-moderate thrombocytopenia groups. Furthermore, a subgroup analysis showed that rescue therapy with rhTPO could rapidly lead to a recovery of the PC, prolong days free from organ support, increase survival days, and reduce the 28-day mortality in sepsis patients with severe thrombocytopenia. These results suggested that sepsis patients with severe thrombocytopenia, not mild-moderate thrombocytopenia, had a poorer prognosis. RhTPO, probably as effective rescue therapy, could quickly recover PC and improve the prognosis in these sepsis patients.

1. Introduction

Sepsis is the most common critical disease with high mortality and is the leading cause of death in the intensive care unit [1]. Based on epidemiological investigations, approximately 15 million people die of infectious diseases every year, accounting for almost 25.5% of the total deaths [2]. The incidence of sepsis in the ICU is increasing year by year, reaching a rate of more than 30% [3,4]. In recent years, despite published treatment guidelines for sepsis, the rapid development of antibiotic therapy, and organ support technologies, the mortality of sepsis patients is still 25% or higher [5]. To improve the prognosis of sepsis patients, it is necessary to distinguish different types of sepsis and develop targeted therapy for certain subtypes.

Clinically, platelet count (PC) frequently altered in sepsis patients and implicated in the pathogenesis of multiorgan failure.

Thrombocytopenia is common in sepsis patients, and the incidence is even as high as 55% [6]. Recent studies confirmed that platelets have a variety of biological functions, not only in hemostasis and thrombosis but also in inflammatory balance in the host, immune responses, tissue repair and regeneration [7–9]. Some clinical studies have shown that thrombocytopenia is closely associated with multiple organ dysfunction syndrome (MODS) and high mortality in sepsis patients [10]. Thiery-Antier N et al found that thrombocytopenia in the first 24 h of septic shock onset was an early prognostic marker for ICU patients [11]. Besides, the patients who had sustained thrombocytopenia or a drop in the PC of greater than 30% during their ICU stay had higher mortality [12].

However, some other authors noted that a drop in the PC may just reflect the severity of sepsis. As we know, platelet change plays an important part in the SOFA score, which is also an independent risk factor for death in sepsis patients [13,14], while the other study

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suggested that thrombocytopenia itself was not associated with increased 28-day mortality in sepsis patients [15]. In addition, some studies also had shown that antiplatelet drugs might reduce the probability of multi-organ failure, hospital stay, and mortality in critically ill patients, including those who were affected by sepsis [10]. Until now, there is insufficient evidence to show a direct causal relationship between thrombocytopenia and mortality. Moreover, recovery of thrombocytopenia might be accompanied by control of infection and improvement of the patient's status, and few studies have explored the impact of platelet-elevating drugs on sepsis-related thrombocytopenia.

To this day, questions regarding this topic remain. Do different degrees of thrombocytopenia make a difference in the prognosis of sepsis patients? Can drug intervention aimed at correcting thrombocytopenia improve the prognosis of sepsis patients? If so, what kind of sepsis patient can benefit from these treatments? In this study, we explored the effect of severe thrombocytopenia on the prognosis of sepsis and the impact of a platelet-elevating drug-recombinant human thrombopoietin (rhTPO) treatment for patients with sepsis-related severe thrombocytopenia.

2. Methods

2.1. Study design and patient identification

This study was conducted as a retrospective two-stage cohort study in the department of intensive care unit (ICU), Shanghai General Hospital, Shanghai, China. The patients were admitted to ICU between September 2015 and October 2018. The diagnosis of sepsis was made by sepsis 3.0 [16].

Inclusion criteria: (1) Age ≥ 18 years and ≤ 80 years, no limitation of sex; (2) Patients admitted to the ICU; (3) Diagnosis or clinical diagnosis of infection; and (4) Changes in Sequential Organ Failure Assessment (Δ SOFA) ≥ 2 .

Exclusion criteria: (1) History of hematopoietic stem cell transplantation or liver, kidney, lung and other solid organ transplantation; (2) History of thrombocytopenia and no response to treatment; (3) Length of ICU stay < 24 h; or (4) Pregnant or lactating patients.

2.2. Definitions

Thrombocytopenia was defined as PC $< 100 \times 10^9/L$, based on the hospital laboratory's reference range. If more than one PC was available within 24 h, the lowest PC was recorded. Degrees of thrombocytopenia were classified into the following categories: normal group (PC $\geq 100 \times 10^9/L$); mild-moderate thrombocytopenia group ($50 \times 10^9/L \leq PC < 100 \times 10^9/L$); and severe thrombocytopenia group (PC $< 50 \times 10^9/L$). Clinical recovery of platelets is defined as PC $\geq 100 \times 10^9/L$ or an increase in PC $\geq 50 \times 10^9/L$ for at least 3 days. The clinical recovery time of platelets was defined as the time to reach clinical recovery.

Definitions of comorbidities: Chronic morbidities were identified if the medical record documented an established diagnosis or if the patient had a known diagnosis or radiologic evidence. In this study, chronic cardiovascular diseases included hypertension, coronary atherosclerotic heart disease, rheumatic heart disease, congestive heart failure, or chronic arrhythmias such as atrial fibrillation; chronic respiratory diseases included chronic obstructive pulmonary disease (COPD), interstitial lung disease, pulmonary tuberculosis, and bronchiectasis; chronic renal diseases included chronic renal insufficiency, chronic nephritis, renal tuberculosis, or nephrotic syndrome; and autoimmune diseases included systemic lupus erythematosus (SLE), rheumatoid arthritis, dermatomyositis, and pemphigus.

2.3. Data collection

The following data were obtained from the patients' electronic

medical records: age, ethnicity, sex, patient type (medical/surgical), comorbidities, and sites and pathogens of infection. Then, clinical characteristic baseline and laboratory results were collected during the first 24 h after enrollment, including markers of organ function and biomarkers of infection (procalcitonin, C-reactive protein, endotoxin). The scores of disease severity during the first 24 h were recorded, including the Acute Physiological and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), and Disseminated Intravascular Coagulation (DIC) scores [17]. The changes in PC within the 14 days were collected. Moreover, the markers of organ function on the 7th day after enrollment were recorded as well. If there were no data on the 7th day, those on the 6th or 8th day could be collected instead. If several pieces of data or measurements were performed at the same time, the lowest/worst data were retained. The serum levels of IL-1 β , IL-2R, IL-6, IL-8, IL-10, and tumor necrosis factor- α (TNF- α) were measured in the first week.

All patients were followed up for 28 days. The following data were also collected from the patients' electronic medical records: number of days free from vasoactive drugs/mechanical ventilation/renal replacement therapy at 28 days, blood product transfusions, survival days at 28 days, and 28-day mortality.

2.4. Statistical analysis

SPSS (Version 20.0. Armonk, NY) statistical software was used for the data analysis. Variables were presented as the means $\pm$ standard deviation (SD) when they were normally distributed. Otherwise, quantitative variables were presented as medians and interquartile ranges (IQRs; 25th and 75th percentiles). Categorical variables were presented as absolute numbers (percentages). The comparisons among normally distributed data were conducted by *t*-test or ANOVA analysis, whereas the Mann-Whitney *U* test or Kruskal-Wallis test was used to analyze non-normally distributed data. Comparisons between the enumeration data were conducted by χ^2 or the Fisher exact method. Survival comparisons between the groups were analyzed by log-rank analysis. $P < 0.05$ was considered to indicate statistical significance. The *P*-value was adjusted when the post-hoc pairwise analysis was conducted. The proportions and survival outcomes were analyzed using Logistic regression or Cox regression including the baseline as the covariate and the group as a fixed factor.

2.5. Ethics

This study was approved by the institutional review board of Shanghai General Hospital (No. 2018KY198). Because the data were anonymous and observational, informed consent was waived. The indication for rhTPO approval by the Chinese Food and Drug Administration (CFDA) did not include sepsis-related thrombocytopenia. In this study, rhTPO was used in only sepsis patients who had severe thrombocytopenia and a high risk of bleeding judged by experienced attending doctors, and written informed consent was obtained from the patients or legal guardians of these patients. Patients in the rhTPO group were treated with rhTPO (TPIAO™, Shenyang Sunshine Pharmaceutical Co., Ltd. Shenyang, China) at a subcutaneous injection dose of 15,000 U/d. The drug treatment was terminated when the PC $\geq 100 \times 10^9/L$, an increase in PC $\geq 50 \times 10^9/L$ lasted for at least 3 days, or severe adverse effects occurred.

3. Results

3.1. Patient characteristics

Between September 2015 and October 2018, 394 patients admitted to the ICU with infection or suspected infection were enrolled. In total, 145 patients were excluded according to the inclusion and exclusion criteria. Finally, 249 sepsis patients were included in the study

Table 1
Demographics and baseline characteristics of sepsis patients.

Characteristic	Normal n = 159	mild-moderate thrombocytopenia n = 41	Severe thrombocytopenia n = 49	P value [†]
Male, n (%)	109 (68.6)	24 (58.5)	29 (59.2)	0.306
Age (years), median (IQR)	64 (52–70)	63 (48–71)	62 (49–70)	0.696
Medical [§] , n (%)	123 (77.4)	34 (82.9)	25 (51.0) ^{*§}	< 0.001
Co-morbidity				
Diabetes mellitus, n (%)	32 (20.1)	6 (14.6)	10 (20.4)	0.711
Cirrhosis, n (%)	4 (2.5)	4 (9.8)	5 (10.2)	0.038
Chronic cardiovascular diseases, n (%)	73 (45.9)	19 (46.3)	20 (40.8)	0.807
Chronic respiratory diseases, n (%)	23 (14.5)	6 (14.6)	6 (12.2)	0.920
Chronic renal diseases, n (%)	13 (8.2)	3 (7.3)	9 (18.4)	0.095
Autoimmune diseases, n (%)	10 (6.3)	3 (7.3)	7 (14.3)	0.194
Sites of infection				
Respiratory tract, n (%)	86 (54.1)	25 (61.0)	14 (28.6) ^{*§}	0.002
Intra-abdominal, n (%)	58 (36.5)	13 (31.7)	29 (59.2) ^{*§}	0.009
Urinary tract, n (%)	10 (6.3)	2 (4.9)	1 (2.0)	0.502
Bloodstream, n (%)	7 (4.4)	4 (9.8)	8 (16.3) [*]	0.020
Skin and soft tissue, n (%)	6 (3.8)	3 (7.3)	3 (6.1)	0.572
Pathogen of infection				
Gram-positive, n (%)	18 (11.3)	8 (19.5)	11 (22.4)	0.105
Gram-negative, n (%)	52 (32.7)	15 (36.6)	21 (42.9)	0.423
Fungi, n (%)	19 (11.9)	5 (12.2)	9 (18.4)	0.499
Virus, n (%)	12 (7.5)	4 (9.8)	1 (2.0)	0.294
Severity of disease				
APACHEII, median (IQR)	14 (10–18)	17 (10–23)	20 (15–24) [#]	< 0.001
SOFA, median (IQR)	5 (3–7)	7 (5–9) [#]	11 (8–13) ^{#§}	< 0.001
DIC score, median (IQR)	3 (2–3)	4 (3–5) [#]	6 (5–7) ^{#§}	< 0.001
The biomarkers of infection				
Procalcitonin (ng/ml), median (IQR)	3.22 (0.64–20.33) n = 150	4.79 (0.61–11.57) n = 41	18.53 (4.05–100.0) ^{#§} n = 47	< 0.001
CRP (mg/L), median (IQR)	162.6 (90.2–247.2) n = 153	140.4 (92.5–198.0) n = 39	142.7 (69.6–242.8) n = 48	0.394
Endotoxin (Eu/ml), median (IQR)	0.063 (0.030–0.140) n = 141	0.053 (0.028–0.136) n = 38	0.060 (0.034–0.185) n = 41	0.794

APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; DIC, Disseminated Intravascular Coagulation; CRP, C-reactive protein.

[§] Medical was as opposed to surgical.

[†] P value was generated by ANOVA or KW method among the three groups.

^{*} Vs normal group, adjusted p < 0.05, [#] vs normal group, adjusted p < 0.001, [§] vs mild-moderate thrombocytopenia group, adjusted p < 0.05.

(Supplementary Fig. 1). In total, 159 patients (63.9%) had no thrombocytopenia, whereas 90 patients (36.1%) had thrombocytopenia: of these, 41 patients (16.5%) had a PC of $50 \times 10^9/L$ to $100 \times 10^9/L$, and 49 patients (19.7%) had a PC of less than $50 \times 10^9/L$. A total of 70 patients (28.1%) died within 28 days.

The demographics and baseline characteristics of the patients are shown in Table 1. There was no significant difference in gender or age among the groups. However, medical patients developed severe thrombocytopenia infrequently. Comorbidities, sites and pathogens of infection among the different groups were largely similar, with a few exceptions. Patients with cirrhosis were more likely to have thrombocytopenia. In addition, compared to the other two groups, patients with severe thrombocytopenia were more often admitted with intra-abdominal or bloodstream infection and less often with respiratory infection. Moreover, sepsis-related thrombocytopenia patients were more severely ill, as reflected by the higher APACHE II, SOFA and DIC scores. Besides, procalcitonin levels were significantly higher in the sepsis-related severe thrombocytopenia group than in the other two groups. However, there was no significant difference between the normal and mild-moderate thrombocytopenia groups. Furthermore, there were no significant differences in C-reactive protein and endotoxin levels among the three groups.

3.2. Sepsis-related severe thrombocytopenia patients had the worse prognosis combined with more blood product transfusions and fewer days free from organ support

Furthermore, the relationship between thrombocytopenia and the prognosis of the sepsis patients was explored. Among the three groups,

there were significant differences in blood transfusion, organ support measures and prognosis of sepsis. Compared with the normal group, the patients with severe thrombocytopenia needed more blood transfusions (platelet, red blood cells, and frozen plasma) and a higher proportion of the blood transfusions, had fewer days free from organ support (vasoactive drug, mechanical ventilation, and renal replacement support), fewer survival days, and higher 28-day mortality. However, there was no significant difference between the normal group and the mild-moderate thrombocytopenia group. The P-value of the post-hoc pairwise comparison was adjusted (Table 2).

After adjusted by age and gender, the patients with severe thrombocytopenia had fewer survival days (hazard ratio, 2.106 (1.259–3.523), p = 0.005) and higher mortality (odds ratio, 2.782 (1.389–5.575), p = 0.004) at 28 days after enrollment. Besides, age was also an independent risk factor for survival days (hazard ratio, 1.053 (1.027–1.080), p < 0.001) and mortality (odds ratio, 1.042 (1.020–1.064), p < 0.001) at 28 days after enrollment. Also, survival at 28 days in the group with severe thrombocytopenia was significantly worse than that in the normal group, according to Kaplan-Meier survival estimates (Supplementary Fig. 2).

3.3. RhTPO could lead to rapid recovery of PC and improve the prognosis of sepsis-related thrombocytopenia

To further explore the effect of severe thrombocytopenia on the prognosis of sepsis patients and find a new effective rescue therapy for sepsis, a subgroup analysis was performed in this study. Of 49 patients with sepsis-related severe thrombocytopenia, 22 patients were treated without any platelet-elevating drugs, whereas 27 patients were treated

Table 2

Patients with sepsis-related severe thrombocytopenia had the worst clinical outcomes among the three groups.

Variable	Normal n = 159	mild-moderate thrombocytopenia n = 41	Severe thrombocytopenia n = 49	P value [†]
Blood product transfusion				
Patients with PLT transfusion, n (%)	6 (3.8)	3 (7.3)	25 (51.0) ^{##}	< 0.001
Patients with RBC transfusion, n (%)	33 (20.7)	8 (19.5)	31 (63.3) ^{##}	< 0.001
Patients with FP transfusion, n (%)	51 (32.1)	15 (36.6)	37 (75.5) ^{##}	< 0.001
Number of PLT transfusion (U), median (IQR)	0 (0–0)	0 (0–0)	1 (0–1) ^{##}	< 0.001
Number of RBC transfusion (U), median (IQR)	0 (0–0)	0 (0–0)	2 (0–5) ^{##}	< 0.001
Number of FP transfusion (U), median (IQR)	0 (0–3)	0 (0–4)	5 (1–14) ^{##}	< 0.001
Organ support				
Days free from vasoactive drugs at 28 days (days), median (IQR)	28 (24–28)	26 (8–28)	23 (7–27) [#]	< 0.001
Days free from MV at 28 days (days), median (IQR)	24 (11–28)	20 (3–28)	11 (0–24) [#]	0.001
Days free from RRT at 28 days (days), median (IQR)	28 (25–28)	28 (14–28)	25 (8–28) [#]	< 0.001
Clinical outcome				
Number of the patients died at 28 days, n (%)	34 (21.4)	15 (36.6)	21 (42.9) [*]	0.006
Survival days at 28 days (days), median (IQR)	28 (28–28)	28 (10–28)	28 (11–28) [*]	0.002

PLT, platelet; RBC, red blood cell; FP, frozen plasma; MV, mechanical ventilation; RRT, Renal replacement therapy.

[†] P value was generated by ANOVA or KW method among the three groups.^{*} Vs normal group, adjusted p < 0.05, [#] vs normal group, adjusted p < 0.001, ^{\$} vs mild-moderate thrombocytopenia group, adjusted p < 0.05.

with rhTPO. There were no significant differences in the patients' demographics or the baselines between the two groups (Supplementary Table 1).

The patients in the rhTPO group had a lower PC on the first day compared with those in the control group, and the increases in the PC within the first 7 days in the rhTPO group were significantly greater than that in the control group. Furthermore, the patients in the rhTPO group probably had a shorter clinical PC recovery time. In addition, 59.3% of the patients in the rhTPO group reached the clinical recovery of the PC in 7 days, compared to 36.4% in the control group, although the difference was not statistically significant. Moreover, the patients in the rhTPO group had longer days free from vasoactive drugs, longer survival days, and lower 28-day mortality compared to the patients in the control group (Table 3).

Compared to the patients in the control group, the patients in the rhTPO group had longer survival days (hazard ratio, 0.204 (0.075–0.554), p = 0.002) and lower mortality (odds ratio, 0.117 (0.023–0.606), p = 0.011) at 28 days after enrollment. The results before and after adjustment by baseline covariables are presented in Table 4. As shown by the Kaplan-Meier survival estimates, the survival outcomes at 28 days in the rhTPO group were better than those in the control group (Fig. 1). Furthermore, no severe drug-related organ dysfunction was observed during rhTPO therapy (Supplementary Table 2).

4. Discussion

In this study, severe thrombocytopenia, not mild-moderate thrombocytopenia, was closely associated with multiple organ dysfunction and higher 28-day mortality in sepsis patients and might play a key role in the prognosis of sepsis. Moreover, for patients with sepsis-related

severe thrombocytopenia, rhTPO, probably as a rescue drug, could restore the PC and improve the prognosis.

Some studies have shown that thrombocytopenia is more likely to occur in sepsis patients with immune system diseases, hematological malignancies, and cirrhosis. Sepsis induced by respiratory infections had a lower incidence of thrombocytopenia, whereas sepsis with bloodstream infections suffered a higher incidence [11,18]. In this study, in addition to similar results, we found that surgical patients were more likely to develop severe thrombocytopenia. There may be several possible reasons: (1) Surgical patients have an increased bleeding risk that results in the loss of platelets; (2) Surgical patients are in a hypercoagulable state to increase the platelet consumption; and (3) The activated coagulation system of the operative site increases platelet demand. In addition, sepsis induced by intra-abdominal infection was related to more severe thrombocytopenia. The liver is the most important organ for thrombopoietin (TPO) synthesis, which is a major platelet-stimulating factor that regulates platelet production. Intra-abdominal infection was more commonly associated with liver and renal dysfunction, which could cause a relative and absolute deficiency of TPO levels in these patients. However, there was no significant difference between the normal and mild-moderate thrombocytopenia groups.

Thrombocytopenia is an important marker that reflects the severity of sepsis [19,20]. In addition, thrombocytopenia can result in dysregulated host inflammatory and immune responses that cause poor outcomes in sepsis patients [21]. De Stoppelaar et al found that thrombocytopenia induced the release of pro-inflammatory factors (TNF- α , IL-6) and an imbalance of the inflammatory response to aggravating the progression of sepsis [22]. Another study found that PC was negatively correlated with intercellular adhesion molecule (ICAM), interleukin-8 (IL-8), and suPAR expression in sepsis patients, and sepsis-related thrombocytopenia was correlated with higher mortality [23].

Table 3

RhTPO could rapidly recover PC and improve the prognosis of patients with sepsis-related severe thrombocytopenia.

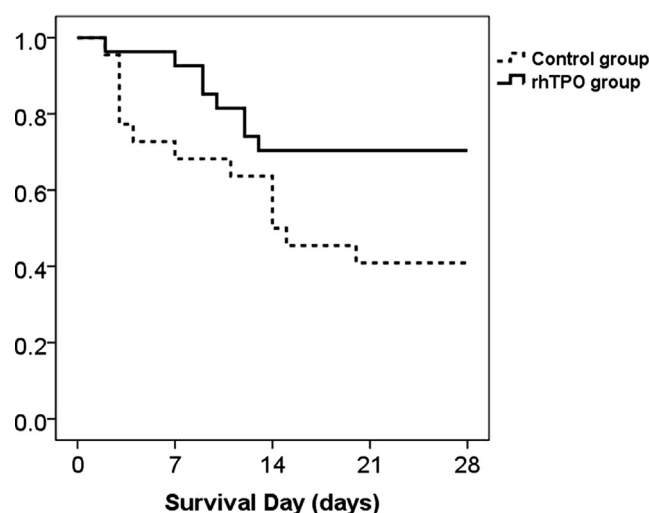
Variable	Control group, n = 22	rhTPO group, n = 27	P value
PLT-1 (10 ⁹ /L), mean (SD)	33 (12)	20 (10)	< 0.001
Δ PLT within the 7 days, median (IQR)	22 (-3–84)	75 (18–126)	0.024
Number of patients with clinical recovery within the 7 days, n (%)	8 (36.4)	16 (59.3)	0.111
Clinical recovery time of PC (days), median (IQR)	24 (7–28)	7 (5–28)	0.018
Days free from vasoactive drugs at 28 days (days), median (IQR)	22 (1–25)	25 (11–28)	0.041
Days free from MV at 28 days (days), median (IQR)	6 (0–19)	17 (1–28)	0.117
Days free from RRT at 28 days (days), median (IQR)	15 (3–28)	25 (13–28)	0.115
Survival days at 28 days (days), median (IQR)	14 (3–28)	28 (13–28)	0.007
Number of the patients died at 28 days, n (%)	13 (59.1)	8 (29.6)	0.038

PC, Platelet count; PLT-1, PC of the patients in the 1st day; MV, mechanical ventilation; RRT, renal replace treatment.

Table 4

RhTPO was an independent protective factor for the prognosis of patients with sepsis-related severe thrombocytopenia.

Variable	Unadjusted			Adjusted ^{&}		
	OR [†] /HR [‡]	95%CI	P value	OR [†] /HR [‡]	95%CI	P value
28-day mortality						
Age	1.011 [†]	0.970–1.055	0.597	1.017 [†]	0.963–1.075	0.547
Male	1.636 [†]	0.516–5.187	0.403	1.203 [†]	0.277–5.223	0.805
APACHE II	1.143 [†]	1.037–1.260	0.007	1.060 [†]	0.898–1.251	0.492
SOFA	1.264 [†]	1.033–1.547	0.023	1.233 [†]	0.828–1.835	0.302
Treated with rhTPO	0.200 [†]	0.059–0.679	0.01	0.117 [†]	0.023–0.606	0.011
Survival days at 28 days						
Age	1.008 [‡]	0.976–1.040	0.631	1.012 [‡]	0.976–1.050	0.504
Male	1.420 [‡]	0.602–3.345	0.423	0.942 [‡]	0.371–2.392	0.901
APACHE II	1.083 [‡]	1.025–1.143	0.004	1.004 [‡]	0.906–1.114	0.937
SOFA	1.188 [‡]	1.049–1.344	0.006	1.259 [‡]	0.999–1.588	0.051
Treated with rhTPO	0.309 [‡]	0.125–0.769	0.012	0.204 [‡]	0.075–0.554	0.002

[†] OR, Odds ratio.[‡] HR, Hazard ratio.[&] OR or HR was adjusted by age, male, SOFA and APACHE II score.**Fig. 1.** The 28-day survival curves of the control and rhTPO groups.

Sepsis-related severe thrombocytopenia could release inflammatory factors, stimulate endothelial cells, inhibit signaling pathways related to leukocyte adhesion/activation, activate complement-related signaling pathways, and cause dysregulated host defense responses [18].

In this study, we found that a greater decrease in PC was associated with higher disease severity scores. Meanwhile, patients with severe thrombocytopenia had higher PCT levels than those with mild-moderate thrombocytopenia and normal groups. In addition, patients with severe thrombocytopenia were more likely to have multiple organ dysfunction and higher levels of serum IL-6 and IL-10, while those with mild-moderate thrombocytopenia showed no significant difference compared with the normal group (Supplementary Tables 3 and Supplementary Table 4). The findings suggested that only severe thrombocytopenia might be closely associated with organ dysfunction and inflammatory imbalance in sepsis patients. Furthermore, patients with severe thrombocytopenia, not mild-moderate thrombocytopenia, had more blood transfusions, fewer days free from organ support and survival days, and higher 28-day mortality compared with the normal groups. These results showed that sepsis-related severe thrombocytopenia rather than mild-moderate thrombocytopenia not only increased platelet transfusion but also led to a higher risk of bleeding or more days of organ dysfunction.

Compared with no thrombocytopenia and mild-moderate thrombocytopenia, patients with sepsis-related severe thrombocytopenia had the worst prognosis, and further study will be needed to explore

targeted therapy for these patients. Some studies showed that sepsis patients with persistent thrombocytopenia had longer ICU and hospital stays and higher 28-day mortality [15]. Therefore, therapy aimed at rapidly correcting the thrombocytopenia may improve the prognosis of certain sepsis patients. Clinically, the most common therapy used to increase the PC is platelet transfusion. However, because of resource scarcity, transfusion-related immune and infectious complications, ineffective transfusion and platelet antibody production, platelet transfusion is limited in clinical practice and has a rigorous indication in sepsis patients [5]. The drugs increasing the PC may improve the prognosis of these sepsis patients.

To explore the impact of rhTPO, which is a platelet-elevating drug for sepsis-related severe thrombocytopenia, the subgroups of severe thrombocytopenia treated with or without rhTPO were analyzed. RhTPO can stimulate the proliferation, differentiation and maturation of megakaryocytes to effectively increase the PC [24,25]. It has been confirmed that rhTPO could effectively increase the PC in patients with immune or chemotherapy-related thrombocytopenia and has no serious side effects [26,27]. In addition, rhTPO was also found to be a potentially effective therapy for patients with ITP during pregnancy, which suggested that rhTPO is a safe drug [28]. However, few studies have explored the effect of rhTPO on sepsis. A retrospective study in senior sepsis patients with thrombocytopenia found that rhTPO could increase the PC, reduce bleeding events, and lead to the recovery of organ function, with no serious adverse events [29]. In perioperative patients with abdominal infection, rhTPO could restore the PC in 76.32% of patients with thrombocytopenia compared with 17.5% of the control group within 5 days and could reduce platelet transfusion and 28-day mortality [30]. However, in these trials, sepsis patients with mild-moderate thrombocytopenia were included. In our study, the impact of rhTPO was explored in patients with severe thrombocytopenia. The findings indicated that rhTPO could rapidly increase the PC, shorten the recovery time of platelets and organ support, prolong survival days, and reduce the 28-day mortality in sepsis-related severe thrombocytopenia. No severe drug-related organ dysfunction during rhTPO therapy was observed. RhTPO may become an effective and safe rescue therapy to improve the prognosis of sepsis-related severe thrombocytopenia. The main limitations of this study were its retrospective, single-center design; the sample of patients with severe thrombocytopenia was small; the patients were non-randomized to the two groups. Besides, this is crucial since macrophage-activation syndrome (MAS) measured by Hemophagocytosis Scores (HScores) is an independent life-threatening entity in early sepsis, and there may be a correlation between severe thrombocytopenia and MAS [31,32]. However, this study was a retrospective clinical study and some parameters of HScores (such as

Ferritin, Triglyceride, Hemophagocytosis features on bone marrow aspirate) had not been tested in this study, so MAS of the patients could not be analyzed. To further confirm this hypothesis, our prospective, multicenter, randomized clinical trial is currently in progress (ClinicalTrials.gov, NCT02707497).

5. Conclusions

Severe thrombocytopenia ($PC < 50 \times 10^9/L$), not mild-moderate thrombocytopenia, is closely associated with poor outcomes in sepsis patients. Additionally, rTPO is likely a rescue therapy that can lead to the quick recovery of the PC and improve the prognosis of patients with sepsis-related severe thrombocytopenia.

CRediT authorship contribution statement

Zhigang Zhou: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Tienan Feng:** Methodology, Software, Formal analysis, Writing - review & editing. **Yun Xie:** Resources, Validation, Data curation. **Xiaoyan Zhang:** Resources, Data curation. **Jiang Du:** Methodology, Software. **Rui Tian:** Investigation, Validation. **Biyun Qian:** Methodology, Project administration. **Ruilan Wang:** Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cyto.2020.155227>.

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